ON *LECUDINA PELLUCIDA* (KÖLLIKER) MINGAZZINI (1891) FROM THE GUT OF *NEREIS CHILKAENSIS* SOUTHERN

By P. N. Ganapati, M.A., D.Sc.

(*From the University Zoological Laboratory, Madras*)

Received January 1946

(Communicated by Dr. N. Kesava Panikkar, F.A.SC.)

CONTENTS

Introduction

While examining the polychætes from the Madras Harbour in search of Sporozoan parasites, I frequently came across a gregarine infecting the gut of *Nereis chilkœnsis*. A perusal of the existing literature soon convinced me that all the previous accounts of gregarines from species of *Nereis* are fragmentary and that there is great diversity of opinion especially regarding the nature of the epimerite, if we are to take for granted that all these accounts refer to one and the same species. Apart from a brief note by Leger (1893), nothing seems to be known about its life-history.

Kölliker as early as in 1848 recorded and figured a gregarine from *Nereis* (unknown species) and called if *Gregarina pellucida*. Lankester (1863) considered *Gregarina pellucida* as a monocystid and described it as *Monocystis nereidis* (pellucida Köll.). Mangazzini (1891) observed the same form and called it *Lecudina pellucida*. Two years later Leger (1893), working independently, created the new genus *Doliocystis* for the species described by Mingazzini. He described two forms, *D. nereidis*, from the intestine of *Nereis cultrifera* (Gr.) and *D. polydoræ*, from the gut of *Polydora agassizi*

Clap. The earlier work of Mingazzini was overlooked by subsequent workers and the name used by Leger came into more frequent use. Labbé (1899) raised the genus to the status of a family, the Doliocystidæ. Brasil (1904, 1909) from an examination of Léger's type species and of others came to the conclusion that *Doliocystis nereidis* is apparently synonymous with *Doliocystis pellucida* (Köll.) and found these parasites living in the gut of *Nereis* (*Lipephile*) *cultrifera* (Gr.). Kamm (1922) considers that both *Ophiodina* Mingazzini and *Doliocystis* Léger are synonymous with *Lecudina* Mingazzini. She created the family Lecudinidæ and defined it as including 'Non-septate Gregarines inhabiting the digestive tract of polychætes Epimerite a simple knob'. Reichenow (1929) amplified and amended. Kamm's definition as follows: 'Body non-septate, distinguishable from the Monocystids by the protoplasm in the anterior portion possessing finer granules. A typical epimerite for attachment to the wall of the gut of the host is present, but may be lost in fully grown individuals. Syzygy does not occur. Sporocysts oval, with a thickening at one pole. Parasites of marine annelids'.

The gregarine in *Nereis chilkœnsis* undoubtedly agrees with the genus *Lecudina* in all its features. As already stated there has been want of agreement in the descriptions of gregarines from species of *Nereis,* even though there are reasons to believe that all the authors apparently refer to one and the same species of parasite, which, under the present accepted system of classification, is referred to as the Genus and type species of the family Lecudinidæ Kamm, and named *Lecudina pellucida* (Köll.) Mingazzini. I have not examined *Nereis cultrifera* and, as such, in drawing any comparison of the present form with the Gregarine recorded from the above host, I have to depend entirely on the meagre descriptions and figures given by previous workers. The epimerite of the gregarine from *Nereis chilkœnsis* is very much like that of *Doliocystis pellucida* (Köll.) Ming. as described and figured by Brasil (1904). The shape of the trophozoite is, however, different and resembles the figure given by Kölliker. Kolliker evidently did not observe the epimerite, but both this author and Mingazzini mention the retractility of the anterior end within the body and in addition they show the protoplasm at the anterior end as nearly transparent and different from the rest of the body. Léger (1893) describes the epimerite in *Doliocystis nereidis* as intracellular and caducous, but Brasi (1904, 1909) who restudied Léger's type species has shown that this is an error, the epimerite being a permanent invaginable sucker-like organ.

Taking for granted that both these authors refer to one and the same parasite, my observations on the gregarine from *Nereis chilkœnsis,* confirm

Brasil's description as regards the nature and structure of the epimerite. I am, therefore, reluctant to create a new species for the form seen by me but instead, propose to retain it as the Genus and type species of the family Lecudinidæ Kamm. The general outline of life-history agrees with the brief description given by Léger for *D. nereidis*, but the spore formation takes place outside the host in sea-water as in *Lecudina brasili* (Ganapati and Aiyar, 1937). It is the purpose of the present paper to give in detail the morphology and life-history of what I consider to be *Lecudina pellucida* (Köll.) Ming. with a view to filling the gaps left by previous observers, and to compare the form with other known species of the genus. In this work I have been fortunate in being able to examine a large quantity of material both living and fixed instead of being content with a few cysts collected at random and with great difficulty as was the experience of earlier authors.

Material and Methods

All the worms examined were collected from the Madras Harbour, where they occur in fairly large numbers in the crevices of stones or creeping on shells of oysters. As soon as they were brought to the Laboratory they were thoroughly cleaned and kept in shallow glass troughs in sea water where they remained healthy for about a fortnight with daily change of water. No worm examined was completely free from the parasite, the nature of infection being only a question of degree. Owing to the large size of the worm and the thickness of its body-wall, it was necessary to cut the worm into bits and to examine the fluid oozing out from the alimentary canal, before deciding whether there are parasites or not. Fresh observations, smears and sections were made adopting the methods described in a previous paper (Ganapati and Aiyar, 1937). Cyst formation takes place in the mid-gut and the cysts are passed out along with the fæcal pellets of the worm. After clearing the debris they were immediately fixed in Bouin-Duboscq's fluid kept at 60° C. in the bath for one hour and at room temperature for 24 hours. Embedding was done in paraffin and sections made 4 to 8 microns in thickness. Heidenhain's hæmatoxylin was used for staining both sections and smears.

The living parasite was observed in a medium of the body-fluid of the host diluted with sea-water. Cysts kept in petri-dishes in a moist chamber completed their development in about 3 days, liberating the spores.

Observations on the Gregarine

The earlier stages of the parasite are intracellular within the epithelial cells of the gut of the host (Photomicrograph 1). They are mostly found in the middle region of the intestine where the epithelium is thick. The

youngest parasite was ovoidal and measured 8 microns along the longer axis (Fig. 1). The cytoplasm is uniformly granular and the pellicle thin. The nucleus which is more or less centrally placed is spherical with a distinct nuclear membrane. There is usually one and in some cases two deeply staining karyosomes closely applied to the nuclear membrane. When there are two, one is considerably larger than the other. The nuclear sap is clear without any chromatin. In sections the parasite appears surrounded by a vacuole within the protoplasm of the host cell. It appears possible that the vacuole is formed at the time of fixation and that in the living condition this space is occupied by a clear fluid which forms the nutriment for the growing parasite and which is absorbed through the thin pellicle covering the parasite.

As growth proceeds one end of the parasite facing away from the gut lumen gets differentiated from the rest of the body. This end which we may hereafter call the anterior end is in the form of a blunt rounded knob (Fig. 2). The cytoplasm at this end is finely granulated and clearly marked out from the general body protoplasm which is coarse consisting of larger granules. The changes mentioned above are visible in parasites 14 microns by 10 microns. At a still later stage when the parasite has attained a length of about 20 microns, it is found attached to the host cell by a short truncated cone-like structure at the anterior end (Fig. 3). This organ of attachment or epimerite persists even after the parasite outgrows the host cell and hangs down into the gut. From observations made on the adult gregarine it is gathered that the attachment is effected by a sucker-like action of the cup-shaped end of the epimerite. It is probable that in addition to being an organ of attachment the epimerite may also function as an organ through which nourishment is absorbed from the host's tissue.

The host cells containing the earlier stages of the parasite are not much differentiated from the surrounding cells. With the growth of the parasite, however, the infected cells undergo hypertrophy, the protoplasm becomes more vacuolar and the nuclei which at first show signs of hypertrophy later on completely degenerate and get pushed to one side. When a large number of parasites are concentrated the epithelium in the region is swollen and projects into the gut lumen (Photomicrograph 1). When the cytozoic phase is over, the host cell is ruptured and the animal hangs down into the lumen of the gut still attached to the epithelium by the epimerite (Fig. 4).

The Trophozoite

The adults or trophozoites occur in large numbers in the middle and posterior region of the intestine (Photomicrograph 2). They are rarely seen in the anterior region. To observe the living parasites the worm was

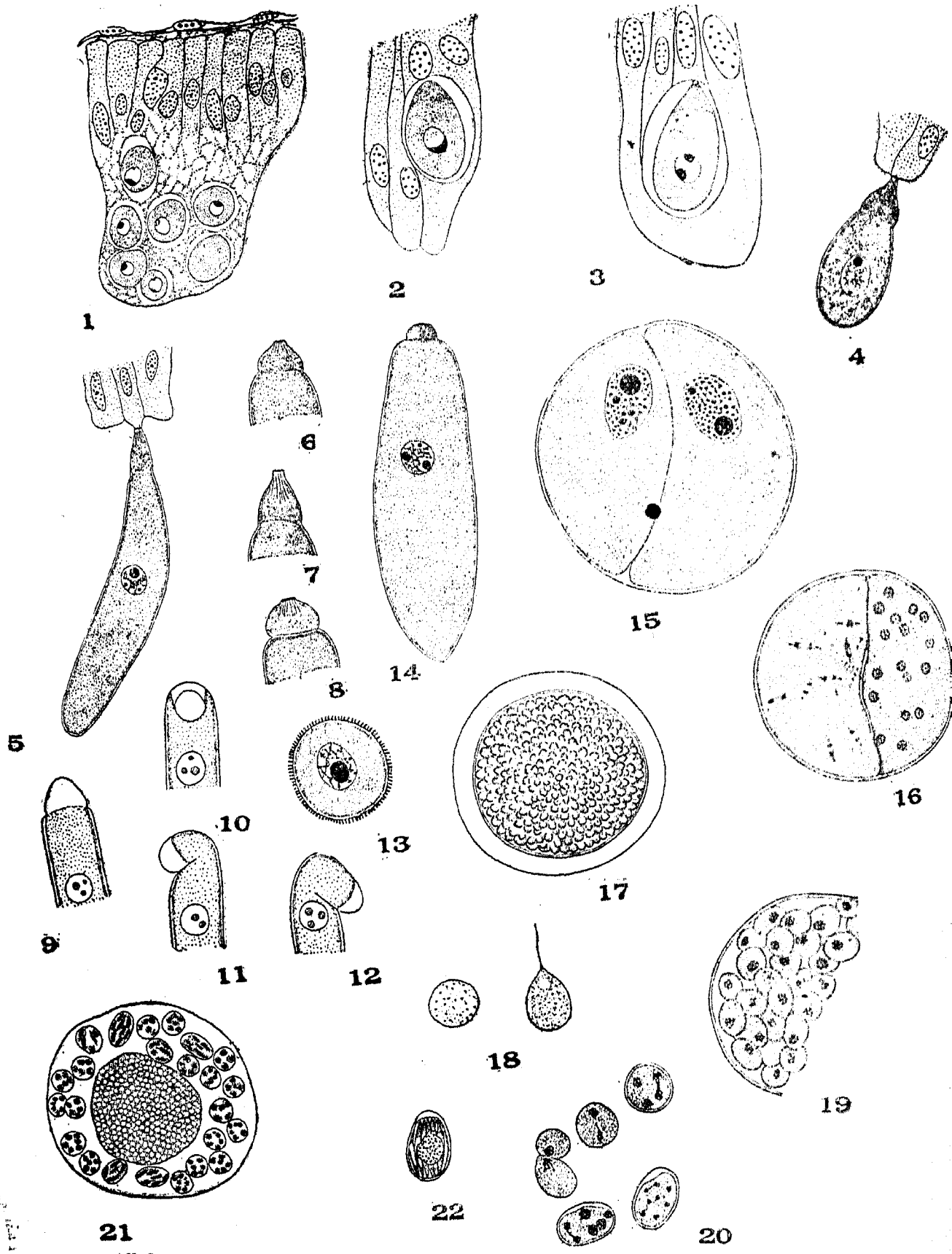
cut into small bits and teased out on a slide with a fine pair of needles. The body fluid of the worm mixed with a drop of sea water served as a very favourable medium for observing the fresh parasites, for a considerable length of time, without any apparent degeneration.

The free trophozoites are elongated and cylindrical with a thick pellicle covering the body (Fig. 5). The cytoplasm is uniformly granular except for a small zone at one end which is transparent and hyaline, clearly marked out from the general body protoplasm. In some instances a clear line or septum separating these two regions is visible but, as explained in a later part of the paper, a true septum is not present, the deceptive appearance being brought about by the sinking in of the anterior region or protomerite into the main body. These two regions, on comparison with the Septate or Cephaline Gregarine, may be called the protomerite and deutomerite respectively. The epimerite is hardly visible in fresh preparations, being almost always in a retracted condition in the free parasite.

The differentiation of the protoplasm at the anterior end from that of the remainder of the body was illustrated both by Kölliker (1848) and Mingazzini (1891). Léger (1893) does not seem to have noted this difference in *Doliocystis nereidis*. Brasil (1904, 1909) clearly brings out this difference in *D. pellucida, D. elongata* and *D. legeri*. I have drawn attention to this difference in *Lecudina brasili* (Ganapati and Aiyar, 1937) and have since found the same feature in two other new forms of *Lecudina* (Ganapati, 1946).

The free trophozoites perform active gliding movements without any change in body shape. The change in shape is mostly confined to the protomerite and a small region of the deutomerite behind it (Figs. 9 to 12). This part of the parasite bends over till the two limbs are nearly parallel and then the animal suddenly straightens itself with a jerk. There is another type of movement in which the protomerite alone takes part. The protomerite is capable of contraction and elongation in an antero-posterior axis and, in addition, it performs a twisting movement from side to side. When the parasite glides along it can be noticed that the protomerite sways from side to side.

The trophozoites attached to the gut epithelium grow till they reach a size of about 100 to 150 microns in length and 20 to 30 microns in width. The body is elongated and cylindrical as seen from sections (Fig. 13). The posterior end is bluntly rounded while, anteriorly, the deutomerite is imperceptibly continued into the protomerite and the epimerite when the animal is in a fully extended condition (Photomicrograph 3). However, as usually seen, there is a waist-like constriction or neck between the protomerite and



All figures were drawn from sections with the aid of a camera lucida at stage level, with Zeiss apochromatic oil immersion objective and compensating oculars. Unless otherwise stated,

the material was fixed in alcoholic Bouin-Duboscq and sections stained in Heidenhain's iron-alum hæmatoxylin. The magnifications given are those at which the drawings were made but Figs. 1–22 have been reduced to half.

Fig. 1. Part of gut showing a few intracellular parasites × 720.
Fig. 2. A slightly advanced intracellular stage in which the protoplasm is differentiated at the anterior end × 1080.
Fig. 3. A still later stage where the epimerite is present × 1080.
Fig. 4. The trophozoite just liberated from the host cell and attached to the gut epithelium × 1080.
Fig. 5. A full-grown trophozoite × 444.
Figs. 6 to 8. The anterior end of the parasite showing different stages in the retraction of the epimerite × 1080.
Figs. 9 to 12. Freehand sketches of living parasites showing the changes of shape assumed by the anterior end. ×
Fig. 13. A cross-section of the trophozoite in the region of the nucleus × 1080.
Fig. 14. A ripe sporont ready for association × 444.
Fig. 15. Cross-section of a cyst × 1080.
Fig. 16. Section of a later cyst × 1080.
Fig. 17. A fresh gametocyst × 480.
Fig. 18. The gametes male and female × 1440.
Fig. 19. Part of section of a gametocyst showing fully formed gametes × 1080.
Fig. 20. Stages in the formation of the sporoblast and sporocyst × 1440.
Fig. 21. Section through a ripe cyst showing sporocysts and crystal residuum × 1080.
Fig. 22. A ripe sporocyst showing sporozoites and sporocyst residuum × 1440.

the main body segment. The nucleus is situated in the anterior half of the deutomerite in the middle of the body. The nucleus is spherical measuring about 10 microns in diameter. There is a distinct nuclear membrane and the nuclear sap is filled with a thick network of chromatin granules. There are 3 to 5 karyosomes of which one is always much larger than the others.

The cytoplasm is coarsely granular in the main body segment and stands in sharp contrast with the finely granulated cytoplasm of the protomerite. The pellicle is thick and is traversed by longitudinal ridges. In transverse sections the trophozoites are circular and the ridges separated by shallow grooves are clearly visible (Fig. 13).

The protomerite is marked out from the deutomerite not only by the difference in the consistency of the cytoplasm in these two regions but also by the capacity of the former to assume different shapes according to its degree of extension or contraction. This difference is markedly evident in living parasites. There is, however, no septum between the two regions as in the Cephaline Gregarines. Often a false septum is seen in fresh parasites brought about by the sinking in of the protomerite into the main body segment. Figs. 6 to 8 explain how this is brought about.

The epimerite is a short conical structure continued directly from the anterior end of the protomerite. The tip of the epimerite by which attachment is effected is cup-shaped and adhesion to the host's tissue is effected by a sucker-like action of this end. The rim of the epimeritic cup stains dark with hæmatoxylin. The whole epimerite is capable of being retracted into the protomerite and in such a condition the parasite appears attached by the protomerite. The pellicle over the main body is fairly thick, but it becomes thin over the protomerite and epimerite. The pellicle in the protomerite and epimerite is traversed by a number of longitudinal striations which appear to be connected with the special contractility of this region.

Both Mingazzini and Kölliker mention the retractility of the anterior end within the body in the forms they studied. Léger, however, describes the epimerite in *Doliocystis nereidis* as 'le segment intracellulaire' which is in the form of a 'simple bouton'. He further states that the epimerite is caducous and that it is left behind when the animal breaks away before attaining the free state. Brasil (1909) who restudied Léger's type species came to the conclusion that the epimerite is not caducous but that it is 'un appareil permanent, invaginable, de la meme categorie que ceaux de *Doliocystis elongata* on de *Lankesteria ascidiæ*'. My observations on the present form has amply substantiated Brasil's findings regarding the nature of the epimerite.

The full-grown trophozoites ripe for association and encystment detach themselves from the gut wall and lie free in the lumen. These mature forms or sporonts differ from the trophozoites in their greater girth as well as the denser consistency of the cytoplasm. The protomerite does not appear to share this increase of girth and it is seen as a blunt knob-like structure at the anterior end (Fig. 14).

Association and Cyst Formation

The ripe sporonts come together in pairs and get attached to each other by their anterior ends. The epimerite serves to hold them together till association is completed. The parasites so attached shorten in length with a proportionate increase in girth. The association becomes lateral. In the next stage they slowly begin to rotate, both together or independently, either in the same direction or in opposite directions. This process goes on for about three quarters of an hour by which time a definite cyst wall, secreted by the activity of the parasites, becomes visible. The cyst formation is completed in about 3 hours. The completed cysts are passed out along with the fæcal pellets of the worm. The cysts were collected at regular intervals

and, after cleaning, fixed for sectioning. It was possible to obtain in this manner cysts showing different stages of gametogenesis and spore formation. When kept in clean sea water or in a moist chamber the cysts completed their development in about 3 days liberating the ripe spores.

A few minutes after the cysts are passed to the outside a thick gelatinous covering makes its appearance around each cyst. This covering or ectocyst is transparent and seems to be formed by the swelling up of a substance on the surface of the cyst wall. The formation of the ectocyst seems to ensure protection against bacteria and other micro-organisms which try to attack the cysts, in their new environment.

The cysts are spherical and ordinarily measures 30 to 40 microns in diameter (Fig. 15). In the fresh condition the cytoplasm of the gametocytes is dense and the partition wall separating them clearly visible.

Gametogenesis and Sporogony

The details of the development of the cyst until the spores are formed can be gathered only from a study of sections. Examination of sections of cysts fixed 2 to 3 hours after they are passed to the outside showed the following changes (Photomicrograph 4). The nucleus has increased in size and the chromatin forms a dense cloud filling the nuclear sap (Fig. 15). The karyosomes have also increased in number up to about 10 and they stain a deep mauve, while the chromatin itself remains bright blue. In the next stage the chromatin forms a highly tangled network or spireme. I have not been able to see completion of the first division. Gametocytes containing four or more nuclei were fairly common and the nuclear divisions take place mitotically. The details of the division are very much the same as those described in *Lecudina brasili* (Ganapati and Aiyar, 1937). Fig. 16 shows an advanced cyst containing resting nuclei in one gametocyte and dividing nuclei in the other. A definite spindle is present but the achromatic figure consists of only a centriole without an aster. When the nuclear divisions are complete, they are seen scattered all over the cytoplasm. Each nucleus is in the form of a few globules of chromatin connected together by a thin network. Just prior to gamete formation there is further condensation of chromatin and the cytoplasm presents a furrowed appearance. The cytoplasm ultimately breaks up into as many bodies as there are nuclei leaving a large spherical mass in the centre, which plays an important role in the dehiscence of the cyst and scattering of spores. Gamete formation is completed in about 48 hours and a fresh cyst examined at this stage presents the appearance shown in Fig. 17.

The fully formed gametes may be liberated by applying gentle pressure on the cover-glass and examined in a medium of sea water. Two types of gametes, presumably produced by the two different gametocytes, are seen. One is more or less circular and inert and the other pear-shaped and motile (Fig. 18). On careful examination, after cutting off most of the light under the microscope, a long slender flagellum performing active lashing movements can be seen, attached to one end of the gamete, which is motile. The movement is forwards in the direction of the flagellum. In sections and smears it is very difficult to make out the flagellum. From a comparison with the gametes in other gregarines it is apparent that the motile type represents the male and the non-motile one the female. Both types contain a few refractile globules in the cytoplasm, which are presumably food material made use of in the formation of the spores. In sections each gamete has a closely alveolated cytoplasm and a nucleus made up of a few large globules of chromatin connected together with a network of the same material (Fig. 19).

Copulation takes place between the two anisogamous gametes and the cytoplasmic fusion precedes nuclear fusion (Fig. 20). The Zygote nucleus divides successively twice forming eight nuclei. The sporoblast membrane which has formed meanwhile becomes thick. In the 8-nucleate stage the cytoplasm inside the sporoblast undergoes longitudinal cleavage resulting in the 8 sickle-shaped sporozoites and the sporoblast membrane becomes the sporocyst wall. As seen from sections the sporozoite nuclei are placed at different levels. A spherical sporocyst residuum is present (Fig. 21).

The sporocysts are ovoidal measuring 9 by 6 microns (Fig. 22). The sporocyst wall presents a characteristic thickening at one pole (Photomicrograph 5). The sporozoites may be liberated by applying gentle pressure on the cover-slip. The living sporozoites exhibit feeble twisting movements. One half of the sporozoite is transparent while the rest of the body is granular. The transparent region represents the nucleus, and in sections the nucleus is seen as an elongated homogeneously staining body.

After spore formation is completed a large quantity of residual protoplasm is left behind in the middle of the cyst. This cystal residuum plays an important part in the dehiscence of the cyst and scattering of the spores by its hygroscopic nature and consequent swelling up by absorption of water through the cyst wall, which by now has become very delicate and weak. I have watched this scattering of spores under the low power of the microscope, the liberated spores appearing as whitish-opaque bead-like bodies.

The Genus Lecudina Mingazzini, 1891

Mingazzini founded the genus *Lecudina* in the year 1891. Two years later Léger (1893) working independently on the species described by Mingazzini, created a new genus *Doliocystis* Léger. The later authors overlooked Mingazzini's work and the name used by Léger came into more frequent use. Kamm (1922) referred back the genus *Doliocystis* Léger as a synonym of *Lecudina* Mingazzini and created the family Lecudinidæ. She also considered that *Ophiodina* Mingazzini is synonymous with *Lecudina* Mingazzini. Kamm (1922) included in the family Lecudinidæ, Gregarines from the intestine of marine annelids having a non-separate body, possessing simple symmetrical epimerites, and producing ovoidal spores presenting an asymmetrical thickening at one pole. A closely allied family the Polyrhabdinidæ was created by Kamm (1922) to include the polycystid (septate) gregarines inhabiting the digestive tract of polychætes and possessing varied epimerites. The family Lecudinidæ Kamm included only a single genus *Lecudina* and under the closely allied family Polyrhabdinidæ was placed three genera, *Polyrhabdina* Mingazzini 1891, *Sycia* Léger 1892, and *Ulivina* Mingazzini 1891. Kamm states that because of the presence of a septum in the Polyrhabdinidæ 'they must be included in the sub-order Cephalina, but that they stand near the borderline with the Acephalina because of their presence only in polychætes'. Reichenow (1929) considers that the presence of a septum in *Polyrhabdina* as described by Kamm is an error, and that the septate appearance is due to the protoplasm in the anterior end of the body being more finely granular. He consequently amalgamated the family Polyrhabdinidæ with Lecudinidæ, and later Reichenow (1932, 1935) included three more genera, *Ancora* Labbe, *Hentschelia* Mackinnon and Ray, *Lecythion* Mackinnon and Ray, in the family Lecudinidæ Kamm as emended by him. Reichenow considers *Sycia inopinata* Léger as identical with *Ulivina elliptica* Mingazzini. Bhatia (1938) is of opinion that *Ulivina* and *Sycia* are distinct as all the three known species of *Ulivina* namely, *U. elliptica* Mingazzini, *U. rhyncoboli* (Crawley) Kamm, and *U. eunicæ* Bhatia and Setna do not show an important generic character of *Sycia*, as given by Léger for the type species, namely a knobbed epimerite bordered at the base by a thick ring. I have described a form (Ganapati, 1946) which undoubtedly belongs to the genus *Sycia* as defined by Leger and possessing a typical epimerite characteristic of the genus. This form does not show a septum and I am inclined to consider that Léger's confusion probably arose from the fact that the body protoplasm at the anterior end is differentiated from the rest of the body. In this respect *Sycia* is as much a dicystid as *Lecudina* and *Polyrhabdina*. It should also be pointed out that Mackinnon and Ray (1931)

Table showing the known species of Lecudina *Mingazzini*, 1891

Name of parasite	Name of Host	Shape of Trophozite	Nature of Epimerite	Measurement in microns	Cysts and Spores	Remarks
1 *L. pellucida* ..	*Nereis culiifera*	Ellipsoidal or bottle-shaped. Protoplasm differentiated at anterior end	Deformable truncated cone ending in a sucker-like cup —Author.	Not given	Cysts small, spores ovoidal. 7 × 5 microns, with thickening at one pole	Triet, Naples and Gulf of Marseilles
Type Species ..	*N. beaucourdrayi*	Protoplasm differentiated at anterior end				
2 *L. leuckarti* ..	*Sagitta* Sp.	Similar to Type Species	..	..	..	..
3 *L. aphroditæ* ..	*Aphrodite aculeata*	..	Long slender 'proboscis'	½" in length	..	..
4 *L. elongata* ..	*Lumbriconereis impatiens*	Elongate-cylindrical	"Piccolo bottone sferico"	500 × 40	..	Intra-cellular stage present
5 *L. heterocephala*	*Nephthys scolopendroides*	Very elongate vermiform. Protoplasm differentiated at anterior end	"A sort of papilla"	..	..	Taken at Naples
6 *L. polydoræ* ..	*Polydora agassizi*	Protoplasm differentiated at anterior end	"D'un tronc de cone a petite base inferieure et il se"	..	..	..
..	*Polydora ciliata*	..				
7 *L.* Sp. ..	*Polymnia nebulosa* *Notomastus exertilis* *Petaloproctus terricole*	..	..	..	..	It is quite possible that several species are involved —Kamm.

8 *L. legeri* ..	*Glycera convoluta*	cylindrical	'A rhizoid filament which penetrates to the base of the host cell'	100 × 25	Cysts small, 45 microns in diameter, spores typical	Mediterranean Coast
9 *L.* Sp. ..	*Polydora socialis*	Spindle-shaped	..	..	..	Not enough data to definitely fix the position of the species
10 *L. brasili* ..	*Lumbriconereis* Sp.	Widest in middle and tapering posteriorly	Slendor prolongation from anterior end ending in an anchor plate	150 × 30	Cysts measure 75 in diameter. Spores 6 × 4 with thickening at one pole	Taken at Madras, India
11 *L. eunicæ* ..	*Eunice siciliensis*	Elongate oval widest in the middle narrowing at both ends	A knob-like structure with its cytoplasm differentiated	475 × 94·5	..	Andaman Islands, Port Blair, INDIA
12 *L. lysidicæ* ..	*Lysidice collaris*	Oval, broadly rounded anteriorly and narrower and pounded off posteriorly. No differentiation of protoplasm at anterior end	A long conical trunk	185 × 62·9 370 × 155·4	..	Andaman Islands, Port Blair, INDIA
13 *L. krusadiensis*	*Platynereis abnormis*	Elongated and cylindrical, protoplasm differentiated at the anterior end	Button-like sucker refractile into the anterior end of the body	100–150 × 20–25	..	Earlier stages are intra-epithelial. Taken at Krusadi Island, Gulf of Mannar, INDIA
14 *L. indica* ..	*Lycastis indica*	Elongated cylindrical body tapering at extremities. Protoplasm differentiated at the anterior end	Cup-shaped depression at anterior end functioning as a sucker	100–190 × 30–50	Cysts spherical measuring 40–70 in diameter. Spores ovoidal 12 × 8 microns	Taken at Madras MADRAS
15 *L. longicephala*	*Pisionideus indica*	Elongate, cylindrical, with anterior end drawn out into a long proboscis	Cup-shaped sucker-like mucron	400–631 × 50	..	Taken at Madras, MADRAS

and self (Ganapati, 1946) have not seen a septum in the species of *Polyrhabdina* studied by us.

Kamm (1922) has assigned an intermediate position for the family Lecudinidæ and Polyrhabdinidæ, between the Acephalina and the Cephalina. This, according to her, is justifiable by the non-septate nature of the body and the possession of an epimerite at the same time. The body, in other words, contains only two segments, the main body representing the protomerite and the deutomerite of tricystids (Cephaline) and an epimerite. Minchin (1912) states that ' it is purely a matter of definition whether these forms be considered as Cephalina without a septum, or as Monocystids with an epimerite and adds that though the terms Dicystida and Tricystida (Cephaline) are to be understood in a purely descriptive sense having no taxonomic value on account of some of the dicystid species having had their origin from tricystid forms secondarily by obliteration of their protomerite, ' such forms as the Doliocystidæ appear to be truly and primitively discystid, and are to be regarded as intermediate forms transitional from Acephalina to Cephalina '.

It may not be out of place to review here in brief a few of the salient features of *Lecudina* which is the best known genus in the family Lecudinidæ Kamm as emended by Reichenow. Of the six genera which have so far been placed in the family, spore formation has been observed only in *Lecudina*. Regarding the others their inclusion in the family can only be provisional (until their sporocyst structure is known).

The early part of the life-history of *Lecudina* is intracellular as observed in *L. pellucida* (Léger, 1893; Ganapati, 1946), *L. elongata* (Brasil, 1909), *L. brasili* (Ganapati and Aiyar, 1937) and a new species of *Lecudina* (Ganapati, 1946). If normally present, and I am inclined to believe it is, this is an important character of the genus, inviting comparison with the Stenophoridæ Léger and Duboscq Cephaloidophoridæ Kamm and Monoductidæ Ray and Chakravarthi, all of which are septate families of gregarines.

The epimerite in all the known species of *Lecudina* is simple symmetrical and deformable, ranging from a simple small papilla in *L. elongata* to a long proboscis ending in a mucron in *L. longicephala* (Ganapati, 1946). In those forms where the epimerite is described as intracellular and caducous, confirmation is lacking and, until this is forthcoming, they may be left out of consideration. In general, it may be stated that the epimerite of *Lecudina* is in marked contrast with the same structure in the large majority of the Cephaline gregarines, where it is a permanent and, in many cases, a complex apparatus which is shed before association and encystment.

The differentiation of the protoplasm at the anterior end from that of the remainder of the body is another feature present in all the species which have been carefully studied; this seems to be characteristic of the genus and according to Kamm ' is an important one in assigning the family Lecudinidæ to an intermediate position between the monocystids and the polycystids'. This differentiation which may be overlooked owing to faulty technique in preservation or staining, cannot be missed in fresh preparations where it is very striking.

The association, cyst formation and spores are known only in four species of the genus and in general it may be stated that the sexual cycle closely resembles and conforms to the polycystid type. While the cyst formation occurs within the host, its further development may take place either within the host or outside in the sea water. In all the three forms studied by me cysts complete their development outside the host. The spores of all the known forms are ovoidal with a characteristic thickening at one pole and they are liberated by simple dehiscence of the cysts.

Summary

1. The detailed morphology and life-history of a dicystid gregarine, *Lecudina pellucida* (Köll.) Mingazzini, from the gut of *Nereis chilkœnsis* Southern, are described.

2. The young stages of the parasite are intra-epithelial while the adult trophozoites lie free in the lumen of the gut attached to the intestinal wall by a sucker-like epimerite.

3. Cyst-formation takes place in the mid-gut and further development of the cysts takes place outside the host.

4. Gametogenesis is completed in about 48 hours. The gametes are anisogamous.

5. Spore-formation takes place in about 3 days and the spores are scattered by simple rupture of the cyst. The sporocysts are ovoidal with a thickening at one pole. There are eight sickle-shaped sporozoites and a spherical sporocyst residuum.

6. The systematic position of the genus *Lecudina* Mingazzini and of the family Lecudinidæ Kamm are discussed in the light of these observations.

Acknowledgments

My thanks are due to Professor R. Gopala Aiyar under whose direction the work was carried out and to the authorities of the University of Madras

who gave the necessary facilities for carrying out the work. I am grateful to Dr. N. Kesava Panikkar for going through the manuscript of this paper before it was sent to the press.

References

Bhatia, B. L. .. "Synopsis of the genera and classification of haplocyte gregarines," *Parasitology*, 1930, 22.

——— .. "Protozoa : Sporozoa," *Fauna of British India*, 1938.

Bhatia, B. L., and Setna .. "On some gregarine parasites from certain polychæte worms from the Andaman Islands," *Proc. Indian Acad. Sciences*, 1938, 8.

Brasil, L. .. "Contribution a la connaissance de l'appareil digestif des annélidesp olychétes," *Arch. Zool. Exper.*, 1904, Ser. 4, T. 2.

——— .. "Le genre *Doliocystis* Léger" *C. R. Acad. Sci.*, 1908, T. 146.

——— .. "Documents sur quelques sporozoaires d'annélides," *Arch. f. Protistenk*, 1909, Bd. 16.

Ganapati, P. N., and Gopala Aiyar, R. "Life-history of a dicystid gregarine *Leuckartina brasili* n. sp., parasititic in the gut of *Lumbriconereis* sp.," *Arch. f. Protistenk*, 1937, Bd. 89.

Ganapati, P. N. .. "Notes on some gregarines from polychaetes of the Madras coast," *Proc. Indian Acad. Sciences*, 1946 (In Press).

Kamm, M. W. .. "Studies on Gregarines, II. Synopsis of the Polycystid Gregarines of the World, excluding those from the Myriapoda, Orthoptera and Coleoptera," *III. Biol. Monogr.*, 1922, 7.

Kölliker, A. .. "Beiträge zur kenntniss niederer Thiere," *Zeits. Wiss. Zool.* 1848, 1.

Labbé, A. .. "Sporozoa ; *Das Tierreich*" 1899, Pt. 5.

Lankester ..

Léger, L. .. "Recherches sur les Gregarines," *Tabl. Zool.*, 1892, 3.

——— .. "L'évolution des gregarines intestinales des vers marins," *C. R. Acad. Sci.* Paris, 1893, 116.

Mackinnon, D. L., and Ray, H. N. "Observations on dicystid Gregarines from marine worms," *Quart. Jour. Micr. Sci.* 1931, 74.

Minchin, E. A. .. "An Introduction to the Study of Protozoa," London, 1912.

Mingazzini, P. .. "Sulla distribuzione delle grégarine policistidée," *Atti. Acc. Lincei, Rend., Roma*, 1891, 7.

——— .. "Grégarine monocistidée, nuove o poco conosciute del Golfo di Napoli," *Ibid.*, 1891, 7.

Ray, H. N. .. "On the Gregarine *Lankesteria culicis* (Ross) in the mosquito, *Aedes* (*Stegomyia*) *albopictus* Skuse. *Parasitogy*, 1933, 25.

Reichenow, E. .. "Protozoa : Sporozoa," *Lehrbuch der Protozoenkunde*, Jena, 1929.

——— .. "Sporozoa" in Grimpe and Wagler, *Tierwelt der Nordund ostee*, Lfg., 1932, 21.

Reichenow, E. .. "Sporozoen" In *Handwurterbuch der Naturwis, enschaften, zweite Auflage*, Jena, 1935.

Setna, S. B. .. "On three new Gregarines, *Bhatiella morphysæ*, *n. g.*, n. sp., *Ferraria cornucephali*, n. g., n. sp., and *Extremocystis dendrostomi* n. g., n. sp., from Indian polychætes," *Rec. Ind. Mus.*, 1931, **33**.

——— .. " Do. A correction," *Ibid.*, 1931, **33**.

Explanation of Plate

Photomicrograph 1. T. S. of gut epithelium showing intracellular stages of the parasite.

„ 2. An adult trophozoite attached to the gut.

„ 3. Section of a cyst soon after it is passed to the outside.

„ 4. Two sporocysts. Note the sporozoites, the sporocyst residuum and the thickening of the sporocyst wall at one pole.

Key to Lettering

intr. par. Intracellular parasite.
ep. Epimerite.
s. Sporocysts.
spz. Sporozoites.
Sp. res. Sporocyst residuum.